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**LOCAL GRAFT VERSUS HOST REACTION  
SUPPRESSED BY IMMUNOBLOCKING ANTIBODY  
SECRETED BY ANTIBODY-FORMING CELLS  
TRANSFERRED WITH THE GRAFT**

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Research was conducted according to the principles enunciated in the  
"Guide for Laboratory Animal Facilities and Care," prepared by the  
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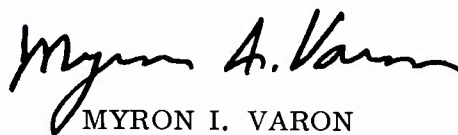
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## FOREWORD

(Nontechnical summary)

Transplantation antigens are the molecular markers on the surface of cells responsible for defining the "tissue type" of an individual in a manner analogous to the way blood group (ABO) antigens define blood type. The immune response to transplantation antigens is mediated both by serum-borne antibodies (humoral response) and small immunocompetent lymphocytes (cellular response). Serum antibodies can inhibit the cellular immune responses when certain special conditions occur naturally or are experimentally produced. For example, it is well established that serum antibody-dependent immunosuppression can result in enhanced growth of tumors and prolonged organ graft survival in animals and man. Both of these phenomena are dependent on alterations in cell-mediated immune responses. Contrary to this, little information is available on antibody-dependent immunosuppression of the graft versus host (GVH) reaction. The GVH reaction occurs when lymphocytes are transplanted into an individual of a foreign tissue type. Control of this reaction is important because it is always seen in experimental or clinical transplantation of foreign bone marrow cells.

In our study, the ability of rat spleen cells to elicit a localized GVH reaction was measured by popliteal lymph node assay. This assay is a measurement of the interaction of grafted cells with host cells of the lymph node located behind the knee. Specific sensitization of the donor to prospective recipient transplantation antigens resulted in diminished GVH reactivity and increased levels of cytotoxic antibody in the serum of the donor. Cytotoxic antibody has been implicated by several workers as the mediator of antibody-dependent immunosuppression.

Using a velocity sedimentation technique that separates cell populations according to size, it was established that sensitized antibody-forming cells from the rat spleen inhibit the GVH reactivity of normal spleen cells. From this observation, we conclude that the immunosuppressive effect of donor sensitization to host transplantation antigens is antibody-dependent and is mediated by antibody-forming cells transferred with the graft.



## ABSTRACT

The graft versus host (GVH) reactivity of Lewis (L) rat spleen cells was measured using a popliteal lymph node (PLN) assay in Lewis-Brown Norway (LBN) recipient rats. Specific sensitization of donors with a single intravenous injection of LBN spleen cells 7 - 14 days prior to assay resulted in a significant decrease in GVH reactivity. Third-party sensitization of donors with Lewis-Buffalo spleen cells did not alter GVH reactivity. L spleen cells suspended in L anti-LBN serum were equally as reactive in the PLN assay as L spleen cells suspended in normal L serum. An association was observed between the degree of immunosuppression as a function of time after sensitization and the titer of cytotoxic antibody in the donor serum. This association was not shown in a study of GVH reactivity and serum cytotoxic antibody titer as a function of the number of cells used. Velocity sedimentation was used to separate spleen cells obtained from specifically sensitized L rats into fractions either enriched or depleted of antibody-forming cells (AFC). The addition of  $10^5$  or  $10^6$  sensitized cells from the AFC-enriched fraction to  $10^7$  normal spleen cells resulted in a significant decrease in GVH reactivity. The addition of  $10^6$  sensitized cells depleted in AFC did not significantly change the GVH reactivity of  $10^7$  normal L spleen cells. From these data we conclude that the suppression in GVH reactivity produced by donor sensitization can be accounted for by immunoblocking antibodies secreted by cells transferred with the graft.

## I. INTRODUCTION

Transfer of a sufficient number of immunocompetent cells into an allogeneic recipient incapable of rejecting them results in a cell-mediated immune response called the graft versus host (GVH) reaction.<sup>10, 39</sup> Prior immunization of the donor against the histocompatibility (H) antigens of the recipient can diminish the intensity of the GVH reaction.<sup>2, 16, 19, 37, 40, 47</sup> The GVH reaction can also be suppressed by treating the recipient with donor antihost serum.<sup>47</sup> Thus, it was postulated<sup>44, 47</sup> that an antibody-dependent phenomenon akin to that described as active enhancement in tumor<sup>23</sup> and organ<sup>18, 24, 34</sup> transplantation experiments is responsible for the reduction in GVH reactivity subsequent to donor sensitization. This view is supported by the observation that lymphoid cells derived from rats bearing long surviving, presumably auto-enhanced renal allografts are less reactive in a GVH assay than cells from normal donors.<sup>2, 40</sup> Tolerance was suggested, however, to explain the decrease in GVH reactivity produced by treatment of donors with solubilized H antigens,<sup>19</sup> and data obtained by measuring the GVH reactivity of lymphoid cells derived from donors incompetent to produce hemagglutinating alloantibodies support this idea.<sup>16</sup>

The purpose of our study was to test the hypothesis that the reduction in GVH activity following donor sensitization is due to active enhancement and, furthermore, that the immunosuppression is mediated by antibody secreted by cells transferred with the graft. To this end, the popliteal lymph node (PLN) assay<sup>15, 26</sup> was used to measure the GVH reactivity of parental rat spleen cells injected into adult F<sub>1</sub> hybrid recipients. Immunization of the donor with an intravenous injection of hybrid spleen cells resulted in a reduction in GVH reactivity. The magnitude of the immunosuppression

depended upon the interval between immunization and assay and was associated with the level of cytotoxic antibody in the serum of the donor. Using velocity sedimentation<sup>29</sup> to separate immunized spleen cells, we were able to show that cells from the fraction enriched in antibody-forming cells (AFC) can inhibit the GVH reactivity of normal spleen cells.

## II. MATERIALS AND METHODS

Alloimmunization of donors. Inbred adult Lewis (L; Ag-B<sup>1</sup>), Lewis-Brown Norway F<sub>1</sub> hybrid (LBN; Ag-B<sup>1</sup>/Ag-B<sup>3</sup>) and Lewis-Buffalo F<sub>1</sub> hybrid (LBuf; Ag-B<sup>1</sup>/Ag-B<sup>6</sup>) rats were obtained from Microbiological Associates, Walkersville, Maryland. The spleens of LBN or LBuf rats were removed, minced in Hanks' balanced salt solution (HBSS) and passed through nylon mesh. The separated cell suspension was then washed twice in HBSS, and the final cell pellet resuspended in fresh HBSS. Viable nucleated cells were counted in a hemocytometer after dilution with 0.2 percent trypan blue. The required number of spleen cells in a volume of 1 ml of HBSS was then injected into the saphenous vein of L rats weighing 150-250 g.

Popliteal lymph node assay. PLN assays were performed as described by Ford et al.<sup>15</sup> Briefly, viable nucleated L or LBN spleen cells were enumerated with the aid of trypan blue. The required number of cells in a constant volume of HBSS (0.1 ml) was injected subcutaneously into the left footpad of LBN rats weighing 60-100 g.

Seven days after injection, the popliteal lymph nodes were dissected and weighed. A PLN index was obtained by dividing the weight of the left lymph node by the weight of the contralateral control node. This transformation of data reduced the variance in GVH activity measured for identical spleen cell populations in replicate assays.

Cell separation. Spleen cell populations from alloimmunized L rats were fractionated by velocity sedimentation, according to the method of Miller and Phillips.<sup>29</sup> The fetal calf serum in phosphate buffered saline gradient was the same as that described for a sheer step linear 15-30 percent gradient. Approximately  $2 \times 10^8$  total cells were loaded onto the gradient for a single run. Cells were collected in 15-ml fractions. All operations were conducted at  $4^{\circ}\text{C}$ . The number of cells in each fraction was measured with the aid of an electronic particle counter (Coulter Electronics, Hialeah, Florida).

Immunization and assay for antibody-producing cells. Sheep erythrocytes (SRBC) were obtained from a single animal and were washed in saline three times before use. For immunization,  $2 \times 10^9$  cells were injected intraperitoneally into L rats. Four days later the number of antibody-producing cells per fraction of sedimented sensitized cells was enumerated by the direct plaque-forming cell (PFC) assay of Pierce et al.<sup>35</sup> with the exception that Seakem agarose was used.

Cytotoxic antibody assay. Chromium-51 release cytotoxic antibody assay was performed essentially as described by Sanderson.<sup>36</sup> Immunized L rats were bled by cardiac puncture, the serum collected, decomplexed at  $56^{\circ}\text{C}$  for 30 min and diluted as needed with HBSS. For target cell labeling, a suspension of Brown Norway spleen cells in HBSS was washed with isotonic  $\text{NH}_4\text{Cl}$  solution<sup>4</sup> to remove erythrocytes, washed three times with HBSS and adjusted to  $1 \times 10^8$  cells/ml. To 0.4 ml of cell suspension, 0.1 ml of a solution of radioactive sodium chromate ( $^{51}\text{Cr}$  sodium chromate, 1 mCi/ml, specific activity 50-500  $\mu\text{Ci}/\mu\text{g}$  of chromium; New England Nuclear, Boston, Massachusetts) was added and the cells incubated for 1 hour at  $23^{\circ}\text{C}$ .

The cells were then washed four times by centrifugation with HBSS, and adjusted to a final concentration of  $5 \times 10^6$  viable cells/ml as determined by trypan blue exclusion. The following substances were added, as listed, to 75 x 100 mm plastic tubes (Falcon Plastics, Los Angeles, California): 100  $\mu$ l of serum, diluted serum, or HBSS to serve as a minimum lysis control; 50  $\mu$ l of reconstituted undiluted guinea pig complement (Microbiological Associates, Bethesda, Maryland); 100  $\mu$ l of labeled spleen cell suspension. The lytic reaction was allowed to proceed at 23°C for 60 min at which time it was quenched by adding 1.0 ml of iced HBSS to each tube. Cells and cellular debris were pelleted by centrifugation and 0.8 ml of supernatant removed for counting in a well-type scintillation counter. Duplicate samples were analyzed and counts between replicate samples rarely varied by more than 5 percent. Cytotoxic antibody titers were expressed as the reciprocal of the highest dilution of antiserum which resulted in activity in the supernatant at least 20 percent greater than that for control samples containing no antiserum.

Statistical analyses. As reported previously,<sup>15</sup> PLN assays must be done at several dose levels in order to perform parallel line analysis leading to a quantitative comparison of GVH reactivity. In general, our experiments were conducted by injecting LBN rats with a uniform number of the spleen cells of interest. Thus, only qualitative comparisons of GVH reactivity were possible. The significance levels of differences between mean PLN indices were determined by the unpaired Student's "t" test. Parallel line analysis was performed on the data obtained in the experiment where the GVH reactivity of normal L spleen cells alone was compared with the reactivity of normal cells mixed with sensitized cells from the velocity sedimentation fraction enriched in AFC.

### III. RESULTS

GVH activity and serum cytotoxic antibody response as a function of time after sensitization. Initial investigations were conducted to establish the kinetics of the immunosuppression of GVH reactivity subsequent to donor sensitization with a single intravenous injection of spleen cells bearing the Ag-B antigens of the prospective recipient. L rats were immunized with a single intravenous injection of  $2 \times 10^8$  LBN spleen cells. At various times thereafter,  $1 \times 10^7$  spleen cells from the immunized rats were injected into the left footpad of LBN assay animals. The PLN indices thus obtained are presented in Figure 1. Also depicted for comparison are the indices obtained by injecting  $1 \times 10^7$  normal L spleen cells and  $1 \times 10^7$  LBN (i.e., syngeneic) spleen cells.

The GVH reactivity of cells from sensitized donors was significantly ( $p < .001$ ) diminished relative to cells from untreated controls on days 7, 10, and 14 after immunization. The maximum depression in GVH reactivity occurred 7 days after immunization. The GVH reactivity of spleen cells from sensitized rats was again normal by 23 days after immunization.

Also presented in Figure 1 are the cytotoxic antibody titers of L rat sera obtained at various times after immunization with a single intravenous injection of  $2 \times 10^8$  LBN spleen cells. The titer was observed to rise to a peak on days 5 - 7 and decline thereafter. Thus, high serum cytotoxic antibody levels were associated with diminished spleen cell GVH reactivity.

#### GVH activity and serum cytotoxic antibody titer as a function of immunizing dose.

To establish if an association similar to that shown in Figure 1 existed between GVH



reactivity and serum cytotoxic antibody titer as a function of sensitizing cell dose, L rats were immunized with various numbers of LBN spleen cells administered in a single intravenous injection. Seven days thereafter,  $1 \times 10^7$  spleen cells from the immunized rats were injected into the left footpad of LBN rats. As evidenced by the

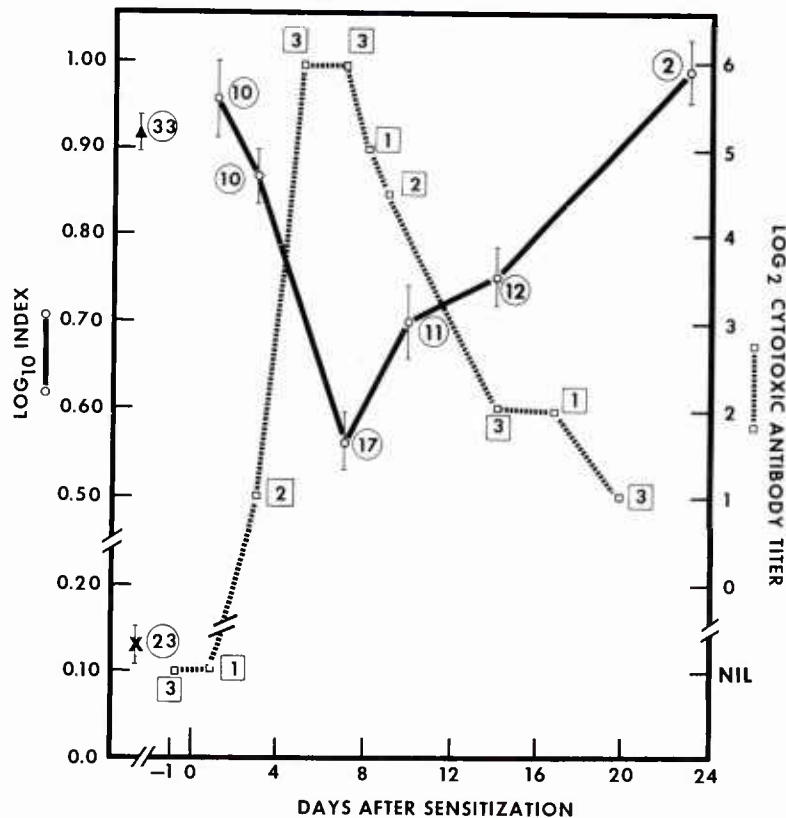


Figure 1. PLN indices (o—o) and donor serum cytotoxic antibody titers (□---□) as a function of the interval between sensitization and assay. Cells from a single L donor were injected into three LBN recipients. Each small circle represents the geometric mean ( $\pm 1$  SE) of the indices obtained by measuring the GVH reactivity of cells from the number of donors indicated within the large circle adjacent to each point. Mean PLN indices were also obtained for normal L cells ( $\blacktriangle$ ) and LBN (syngeneic) cells ( $\times$ ). Each small square represents the geometric mean of antibody titers determined in replicate assays of pooled serum. Serum from two identically sensitized rats was used per assay. The number of replicate assays performed is indicated within the large square adjacent to each small square.

PLN index data presented in Figure 2, the immunosuppressive effect of sensitization was greater when larger doses of LBN spleen cells were administered to prospective donors. On the other hand, the cytotoxic antibody titer of sera obtained from L rats 7 days after immunization with LBN cells was essentially unchanged over the dose range studied.

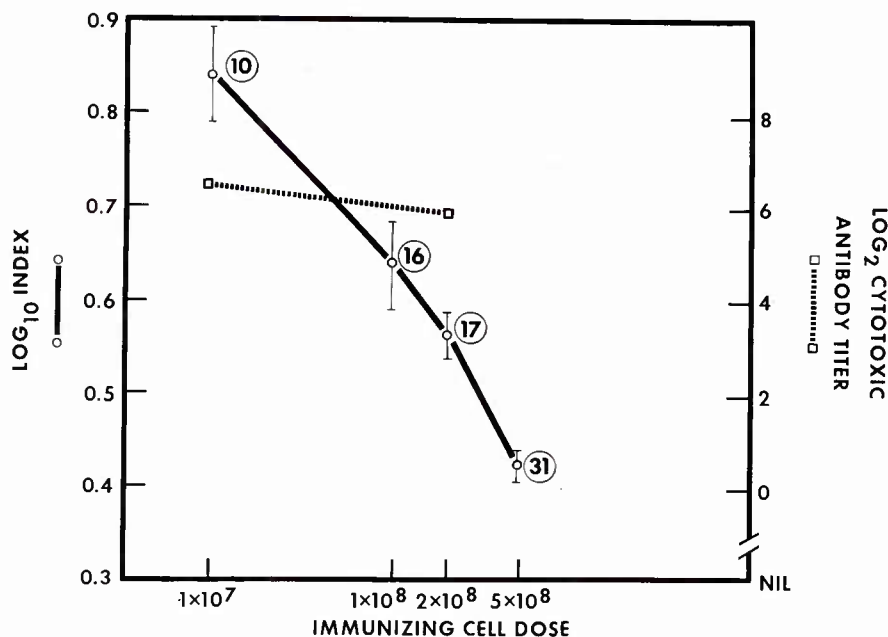


Figure 2. PLN indices (o—o) and serum cytotoxic antibody titers (□---□) as a function of immunizing dose. Cells from a single L donor were injected into three LBN recipients. Each small circle represents the geometric mean ( $\pm 1$  SE) of the indices obtained by measuring the GVH reactivity of cells from the number of donors indicated within the large circle adjacent to each point. Each square represents the geometric mean of three replicate assays of pooled serum from two identically sensitized rats (i.e., six rats total).

Specificity of the immunosuppression. To determine if the immunosuppression resulting from donor sensitization was antigen specific, L rats were treated with a single intravenous dose of either  $5 \times 10^8$  LBN (specific) or  $5 \times 10^8$  LBuf (third party) spleen cells. Seven days later,  $1 \times 10^7$  spleen cells from the immunized rats were



injected into the footpads of LBN recipients and PLN indices thereby obtained. As is presented in Table I, spleen cells derived from rats immunized with third-party cells were not more reactive ( $p > .05$ ) than cells derived from untreated donors, whereas cells derived from specifically immunized rats were significantly ( $p < .001$ ) less reactive. Thus, the GVH hyporeactivity induced by donor sensitization is antigen specific.

Table I. PLN Indices Obtained by Injecting LBN Rats with Spleen Cells Derived from L Rats Sensitized to LBN or LBuf Antigens

	Number of donors (recipients)	Mean $\log_{10}$ index $\pm$ SE
LBuf $\rightarrow$ L $\rightarrow$ LBN	3 (8)	1.026 $\pm$ .065
LBN $\rightarrow$ L $\rightarrow$ LBN	32 (94)	0.430 $\pm$ .021
L $\rightarrow$ LBN	33 (97)	0.918 $\pm$ .020

L rats were sensitized with an intravenous injection of  $5 \times 10^8$  spleen cells of the appropriate strain. The interval between sensitization and PLN assay was 1 week. Recipients were injected with  $1 \times 10^7$  L spleen cells. The cells from each donor were injected into two to three recipients.

GVH reactivity of cells suspended in immune serum prior to inoculation. To establish whether the GVH reactivity of normal spleen cells is inhibited by immune serum transferred with the graft, pooled spleen cells from untreated L rats were suspended in either L anti-LBN or normal L serum prior to injection into LBN footpads. Inoculations consisted of  $1 \times 10^7$  L cells in 0.1 ml of the appropriate undiluted serum. Antisera were obtained from L rats immunized 7 days previously with an intravenous injection of  $5 \times 10^7$  LBN spleen cells. The PLN indices obtained by injecting LBN

rats with L spleen cells suspended in normal or immune serum were not significantly ( $p > .05$ ) different (Table II).

Table II. PLN Indices Obtained by Injecting LBN Rats with Normal L Spleen Cells Suspended in Normal L or L Anti-LBN Serum

Serum	Number of donors (recipients)	Mean $\log_{10}$ index $\pm$ SE
Normal L	2 (6)	0.800 $\pm$ .0789
L anti-LBN	2 (6)	0.996 $\pm$ .061

Spleen cells derived from a single donor were suspended in either normal L or L anti-LBN serum and injected into three recipients with each serum

Velocity sedimentation profile of rat spleen cells. For experiments designed to test for suppression of GVH reactions by antibodies secreted in situ, it was necessary to establish the region of the velocity sedimentation profile for rat spleen cells which is enriched in antibody-forming cells (AFC). Thus, velocity sedimentation was performed on a suspension of spleen cells derived from L rats immunized 4 days previously with SRBC. A typical profile is presented in Figure 3. The populations of cells in tubes 3 - 9 were enriched in PFC. Hence, in subsequent experiments, the pooled content of these tubes was designated as the fraction enriched in AFC, the assumption being made that the cells eliciting alloantibodies appear in a similar region of the sedimentation profile as the cells producing anti-SRBC hemolysins. Tubes 13 - 15 contained relatively few PFC and, for subsequent experiments, the pooled contents of these tubes were designated as the fraction depleted of AFC.

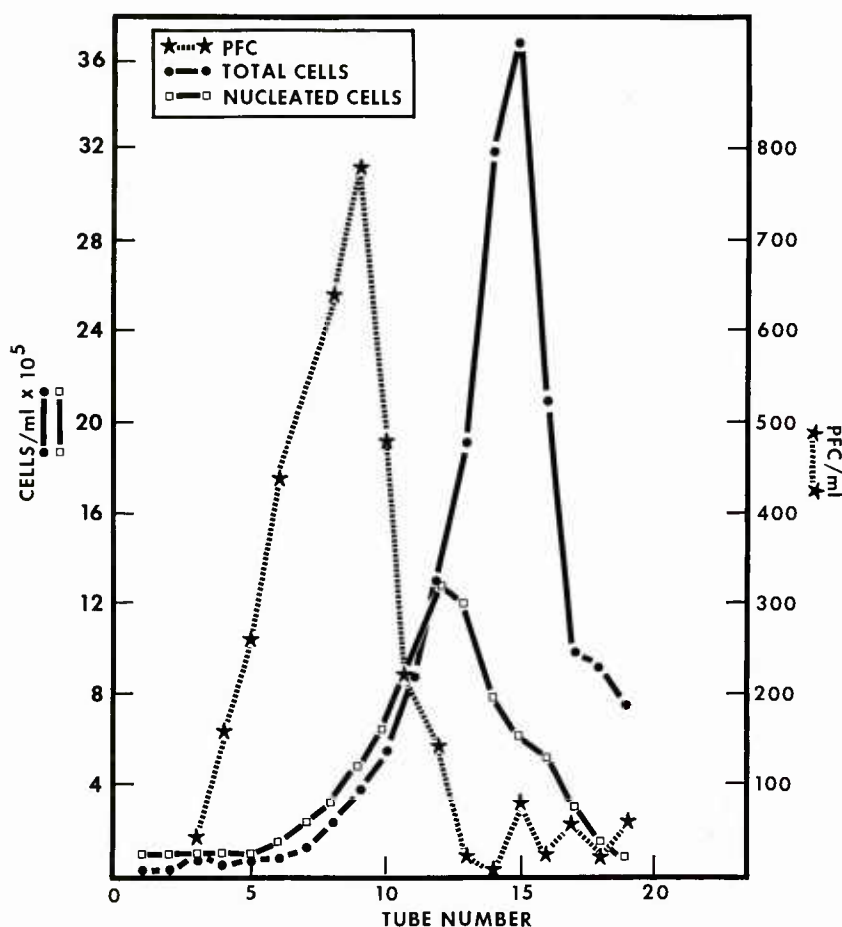


Figure 3.  
Velocity sedimentation  
profile of L rat spleen cells

GVH reactivity of normal spleen cells mixed prior to inoculation with sensitized AFC. Bildsøe et al.<sup>2</sup> suggested that the diminished GVH reactivity of spleen cells derived from rats bearing long-surviving renal allografts might be due to immunoblocking antibodies secreted by cells transferred with the graft. Presumably, a similar mechanism could explain the immunosuppression resulting from the treatment of donors with cells bearing the Ag-B antigens of the prospective recipient. To test this hypothesis, velocity sedimentation was used to separate sensitized rat spleen cells into fractions either enriched or depleted in AFC. Sensitized spleen cells were derived from L rats treated 7 days previously with a single intravenous injection of  $5 \times 10^8$  LBN

spleen cells. Presented in Figure 4 are the PLN indices obtained by injecting LBN rats with  $1 \times 10^7$  normal L spleen cells mixed prior to inoculation with various numbers of sensitized cells from the fraction enriched in AFC. Addition to the inoculum of  $10^5$  or  $10^6$  sensitized cells from the fraction enriched in AFC resulted in significant ( $p < .01$ ,  $p < .001$ , respectively) decreases in the PLN index relative to the value obtained by the injection of only normal L spleen cells. The mean  $\log_{10}$  PLN indices obtained by the injection of  $1 \times 10^7$  normal L cells mixed with  $10^2 - 10^6$  sensitized spleen cells depleted of AFC varied between 0.690 and 0.860. These mean values

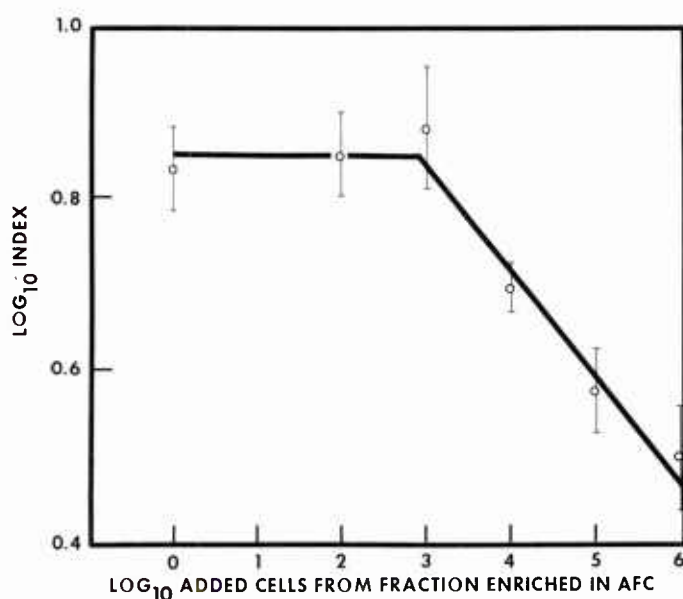


Figure 4. PLN indices obtained by adding sensitized spleen cells from the fractions enriched in AFC to normal L spleen cells prior to injection into LBN rats. Each circle represents the geometric mean ( $\pm 1$  SE) of the indices obtained in two replicate experiments. For each experiment, spleen cells derived from two to three identically sensitized L rats were separated by velocity sedimentation and various numbers of cells from the desired fraction mixed with  $1 \times 10^7$  normal L spleen cells prior to injection. Three or four recipients were used per point per experiment. As a control for each experiment three or four LBN rats were injected with only  $1 \times 10^7$  normal L spleen cells. The geometric mean ( $\pm 1$  SE) of the indices thus obtained is represented by a square.

were the result of two replicate experiments with three or four LBN rats per point. None of the indices differed significantly from the control value obtained by the injection of only normal L cells.

Quantitative estimate of AFC-mediated immunosuppression. To quantitatively estimate the degree of immunosuppression resulting from the addition of sensitized AFC to an inoculum of normal cells, graded doses of normal L spleen cells mixed prior to inoculation with sensitized cells from the fraction enriched in AFC were injected into the footpads of LBN rats. For comparison, graded doses of only normal L spleen cells were also injected into LBN rats. As evidenced by the data presented in Figure 5, the relationship between the logarithm of the PLN index and the logarithm of cell dose for normal spleen cells was linear, as previously observed by others.<sup>15</sup> Addition of sensitized cells from the fraction enriched in AFC to the inocula resulted in PLN indices uniformly lower than those resulting from inocula consisting of only

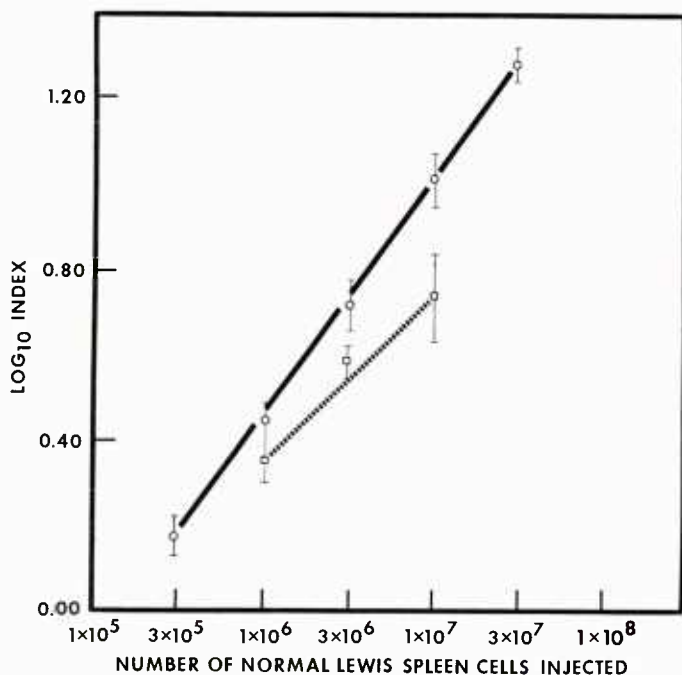


Figure 5.  
Quantitative estimation of AFC-mediated immunosuppression. The data depicted represent the mean of indices obtained in two replicate experiments. Each experiment consisted of injecting LBN rats with various numbers of both normal L spleen cells (o—o) and normal L cells mixed with 10 percent of that number of sensitized cells from the velocity sedimentation fraction enriched in AFC (□---□). Each point represents the geometric mean of three to eight indices obtained by injecting recipients with cells from two to four donors.

normal spleen cells. The slope of the dose-response line for the inocula containing sensitized AFC was not parallel ( $p < .05$ ) to the reference line and thus prevented a meaningful quantitative comparison by parallel line analysis with the dose-response relationship for normal cells.

#### IV. DISCUSSION

Our studies showed that spleen cells derived from donors treated with an intravenous injection of cells bearing the histocompatibility antigens of the prospective recipient were temporarily less competent to initiate a local GVH reaction than normal spleen cells (Figures 1 and 2). These results confirm earlier reports of diminished GVH reactivity following donor sensitization by a variety of other procedures.<sup>2,16,19,37,40,47</sup> The conclusion that the immunosuppression was antigen specific is supported by the observation that spleen cells derived from L rats immunized with LBuf cells were as competent as normal cells to initiate a GVH reaction in LBN rats (Table I).

The hypothesis that this immunosuppression is due to active enhancement mediated by antibodies secreted by cells transferred with the graft is supported by four lines of evidence. These are as follows:

First, our studies showed that antibody-forming cells derived from specifically immunized donors can inhibit the reactivity of normal spleen cells in a local GVH assay. Injecting LBN rats with  $10^7$  normal L spleen cells mixed with  $10^5$  or  $10^6$  sensitized spleen cells from the velocity sedimentation fraction enriched in AFC resulted in PLN indices lower than those resulting from the injection of only normal spleen cells (Figures 4 and 5). The possibility that this immunosuppression was an artifact produced by the cell separation procedure is ruled out by the observation that the

addition of a similar number of sensitized cells depleted in AFC to an inoculum of normal spleen cells did not result in a similar depression in GVH reactivity.

Second, the kinetics of the immunosuppression observed here is similar to that previously reported for enhancement resulting from immunization with viable cells. In our study, GVH reactivity was significantly depressed on days 7 - 14. Similarly, Ockner et al.<sup>33</sup> found that active enhancement of renal allografts was most effectively obtained with an interval of 6 - 13 days between intravenous immunization of recipients with viable cells and transplantation. Enomoto and Lucas<sup>11</sup> found that the peak level of antibody capable of passively enhancing renal allografts occurred on days 3 - 8 after a single intravenous injection of allogeneic cells.

Third, diminished GVH reactivity was associated with elevated levels of cytotoxic antibody in the serum of the donor. This association may be indicative of enhancement because several reports showed that alloantisera<sup>17, 28</sup> or antibodies<sup>22, 41, 42</sup> with complement-dependent cytotoxic activity are also capable of suppressing the cell-mediated immune response. It must be pointed out that contradicting evidence<sup>25, 45, 46</sup> precludes certainty that cytotoxicity and enhancement are mediated by the same classes or subclasses of antibody. Thus, we recognize that the association of elevated cytotoxic antibody levels with decreased reactivity of spleen cells in the PLN assay may be only coincidental and the evidence reported here does not establish a causal relationship. Interpretation of the data as coincidence may be supported by our finding that serum antibody levels remained essentially constant despite a twentyfold increase in sensitizing cell dose, whereas the GVH reactivity of spleen cells declined significantly (Figure 2). Biesecker,<sup>1</sup> however, recently reported that the in vitro cytotoxicity



of sensitized rat lymphoid cells decreased as the dose of immunizing cells was increased from  $10^7$  to  $10^8$ . Similar findings were also reported in mice.<sup>7</sup> Thus, the dose-response data reported here may represent a consistent level of antibody-mediated immunosuppression and a decreasing degree of sensitization of the T cells responsible for initiating the GVH reaction.

The last line of evidence derives from data reported by others. Voisin et al.<sup>47</sup> showed that recipients passively immunized with antihost serum are less susceptible to GVH disease than untreated animals. Hence, the possibility that GVH reactions can be inhibited by alloantibodies was established. Furthermore, support for the hypothesis that enhancing antibodies can be secreted after transfer of cells derives from the reports by Field et al.<sup>12,13</sup> that  $F_1$  hybrid rats that recover from GVH disease are resistant to rechallenge by parental strain lymphoid cells even if they have been rendered immunoincompetent by thymectomy and irradiation. This refractoriness to GVH is evidently mediated by a humoral factor secreted by donor cells since it can be transferred to normal recipients by the cross circulation of blood irradiated with 1500 rads. Additionally, Cerottini et al.<sup>8</sup> found that spleen cells grafted into irradiated allogeneic mice produced alloantibody-forming cells as well as effector cells capable of mediating in vitro cytotoxicity.

Interestingly, host antidonor antiserum was not immunosuppressive when mixed with normal L cells prior to inoculation into the footpad of LBN rats (Table II). A similar observation was reported by Mullen et al.<sup>32</sup> and mentioned by Ford and Simon-<sup>16</sup> The greater immunosuppressive effect of antibody secreted in situ possibly reflects higher local antibody concentrations in the lymph node during the week-long



assay period. This hypothesis is supported by our observation that suppression of the GVH reactivity of normal spleen cells is more pronounced when a larger number of sensitized AFC are present in the graft (Figure 4). Furthermore, Brunner et al.<sup>5</sup> showed that the in vitro cytotoxic activity of sensitized mouse spleen cells was more effectively inhibited by higher antiserum concentrations.

Our results are not sufficient to establish if diminished GVH reactivity resulting from donor sensitization is entirely due to immunoblocking antibodies secreted after transfer. It is conceivable that the T lymphocyte population of the rat spleen is rendered specifically hyporeactive by exposure to alloantigens and that blocking antibodies secreted in situ only further diminish the intensity of the GVH reaction. Such a conclusion might be warranted by reports that the activity of lymphoid cells in the mixed lymphocyte interaction (MLI) is specifically diminished for cells derived from rats either bearing long-surviving renal allografts<sup>40</sup> or sensitized with an intravenous injection of allogeneic lymphoid cells.<sup>43, 48</sup> But the MLI measures the competence of cells to proliferate, whereas assays for GVH activity such as the PLN assay and the Simonsen splenomegaly assay<sup>39</sup> measure the competence of T lymphocytes to damage tissue. This is so because these assays depend primarily upon the proliferation of host cells in response to the immunologic insult rendered by the grafted cells.<sup>3, 20, 21</sup> Thus, cell-mediated cytotoxicity assays possibly provide a better in vitro analogue to GVH reactions than does the MLI. In these assays, it is well established that prior in vivo exposure of lymphocytes to strongly allogeneic H antigens promotes the formation of cytotoxic effector cells.<sup>5, 6</sup> Hence, an interesting parallel to our experiments is the observation by Möller<sup>30</sup> that sensitized spleen cells, probably secreting an

immunoblocking antibody,<sup>44</sup> inhibit the in vitro cytotoxicity of sensitized mouse lymph node cells.

According to our hypothesis, then, exposure of donors to the H antigens of the prospective recipient results in two antagonistic effects characteristic of active enhancement, namely: heightened competence of T cells to mediate cellular immunity, and production of a humoral antibody which inhibits cell-mediated immunity. Hence, if antibodies secreted after transfer are responsible for the diminished GVH reactivity of spleen cells derived from immunized donors, then the small increases in GVH activity observed by Simonsen<sup>38, 39</sup> and others<sup>9, 14, 16, 27</sup> following donor sensitization against strong H antigens may result from a similar mechanism. That is, sensitization results in increased competence of donor T cells to initiate a GVH reaction that is largely masked by the production of blocking antibodies secreted after transfer of the inoculum to the host. Möller<sup>31</sup> proposed a similar explanation that suggested inhibition by a serum factor in the donor before transfer. Clearly, these proposals are not mutually exclusive. Moreover, both are difficult to reconcile with the finding of Ford and Simonsen<sup>16</sup> that prior immunization against a major H antigen results in only small increases in GVH reactivity even for cells derived from a donor rendered incapable of an antibody response by x irradiation.

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13. ABSTRACT <p>The graft versus host (GVH) reactivity of Lewis (L) rat spleen cells was measured using a popliteal lymph node (PLN) assay in Lewis-Brown Norway (LBN) recipient rats. Specific sensitization of donors with a single intravenous injection of LBN spleen cells 7-14 days prior to assay resulted in a significant decrease in GVH reactivity. Third-party sensitization of donors with Lewis-Buffalo spleen cells did not alter GVH reactivity. L spleen cells suspended in L anti-LBN serum were equally as reactive in the PLN assay as L spleen cells suspended in normal L serum. An association was observed between the degree of immunosuppression as a function of time after sensitization and the titer of cytotoxic antibody in the donor serum. This association was not shown in a study of GVH reactivity and serum cytotoxic antibody titer as a function of the number of cells used. Velocity sedimentation was used to separate spleen cells obtained from specifically sensitized L rats into fractions either enriched or depleted of antibody-forming cells (AFC). The addition of <math>10^5</math> or <math>10^6</math> sensitized cells from the AFC-enriched fraction to <math>10^7</math> normal spleen cells resulted in a significant decrease in GVH reactivity. The addition of <math>10^6</math> sensitized cells depleted in AFC did not significantly change the GVH reactivity of <math>10^7</math> normal L spleen cells. From these data we conclude that the suppression in GVH reactivity produced by donor sensitization can be accounted for by immunoblocking antibodies secreted by cells transferred with the graft.</p>			